

Development of a metabolically active, non-replicating sporozoite vaccine to prevent *Plasmodium falciparum* malaria

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Immunization of volunteers by the bite of mosquitoes carrying radiation-attenuated *Plasmodium falciparum* sporozoites protects greater than 90% of such volunteers against malaria, if adequate numbers of immunizing biting sessions and sporozoite-infected mosquitoes are used. Nonetheless, until recently it was considered impossible to develop, license and commercialize a live, whole parasite *P. falciparum* sporozoite (PfSPZ) vaccine. In 2003 Sanaria scientists reappraised the potential impact of a metabolically active, non-replicating PfSPZ vaccine, and outlined the challenges to producing such a vaccine. Six years later, significant progress has been made in overcoming these challenges. This progress has enabled the manufacture and release of multiple clinical lots of a 1st generation metabolically active, non-replicating PfSPZ vaccine, the Sanaria™ PfSPZ Vaccine, submission of a successful Investigational New Drug application to the US Food and Drug Administration, and initiation of safety, immunogenicity and protective efficacy studies in volunteers in MD, US. Efforts are now focused on how best to achieve submission of a successful Biologics License Application and introduce the vaccine to the primary target population of African children in the shortest possible period of time. This will require implementation of a systematic, efficient clinical development plan. Short term challenges include optimizing the (1) efficiency and scale up of the manufacturing process and quality control assays, (2) dosage regimen and method of administration, (3) potency of the vaccine, and (4) logistics of delivering the vaccine to those who need it most, and finalizing the methods for vaccine stabilization and attenuation. A medium term goal is to design and build a facility for manufacturing highly potent and stable vaccine for pivotal Phase 3 studies and commercial launch.

Introduction

Pronouncements have been made for more than 25 years that the development and implementation of a highly effective malaria vaccine is imminent. In the case of pre-erythrocytic stage malaria vaccines, these hopes have been based largely on the observation that immunization of volunteers by the bite of mosquitoes carrying radiation-attenuated, live *Plasmodium falciparum* sporozoites induces immune responses that completely prevent infection upon challenge with fully virulent sporozoites.¹⁻¹²

Live, attenuated, infectious agent vaccines represent about 50% percent of currently licensed vaccines,¹³ providing a record of safety and efficacy over many years and billions of doses. In the eighteenth century Jenner demonstrated the protective effect of vaccination with cowpox infection against smallpox.¹⁴ Numerous successful vaccines were subsequently developed, despite minimal understanding of the biological mechanisms and protein and epitope targets of protective immunity. These successes include the development and widespread deployment in the twentieth century of attenuated vaccines against viruses (e.g., polio, measles, mumps rubella, yellow fever, Japanese encephalitis), and bacteria (e.g., the BCG vaccine for tuberculosis,¹⁵ cholera and typhoid fever^{16,17}).

In 2002 we updated and summarized the published literature on immunizing humans by the bite of radiation-treated *Anopheles* mosquitoes infected with *P. falciparum* sporozoites.¹² Fourteen volunteers were immunized by the bite of greater than 1,000 *P. falciparum* sporozoite-infected, irradiated mosquitoes during a minimum of 5 and a maximum of 19 immunizing mosquito biting sessions (median = 9.5). When challenged by the bite of five non-irradiated *P. falciparum*-infected mosquitoes 2–10 weeks after their final primary immunization, 13 of the 14 volunteers (93%) were protected against asexual erythrocytic stage infection with *P. falciparum*. Six of the volunteers were re-challenged a total of 15 times within 2–10 weeks of final primary (n = 1) or secondary (n = 5) immunization and all six volunteers were entirely protected in all 15 challenges (100%). Six volunteers were challenged between 23 and 42 weeks (weeks 23, 36, 36, 39,

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41 and 42) after final primary (n = 1) or secondary immunization (n = 5), and five of the six volunteers were protected, including the volunteer challenged at 42 weeks. In total 35 challenges were performed in these volunteers and there was complete protection against *P. falciparum* infection in 33 of the 35 challenges (94%). These challenges were done primarily with isolates of *P. falciparum* identical to those used to immunize the volunteers. In four volunteers, seven challenges were also done with heterologous parasite strains. The parasites used for immunization and challenges originated from geographically distinct locations. Nonetheless, all of the volunteers (100%) were protected against the heterologous strain of *P. falciparum*. A dose response effect for this type of immunization was apparent: of 8 volunteers immunized with fewer than 950 immunizing bites and challenged with homologous isolates, only two (25%) were protected.

From the time of the publications of results of the first studies immunizing humans by the bites of irradiated mosquitoes infected with *P. falciparum* sporozoites in the early 1970s,¹⁻⁸ it has been essentially universally recognized that immunization of humans with radiation-attenuated *P. falciparum* sporozoites proved the principle that it was possible to immunologically protect humans against malaria, and provided a gold standard for malaria vaccine efficacy. However, this insight was accompanied by an equally universal consensus that it was inconceivable to consider developing an attenuated *P. falciparum* sporozoite vaccine that would be similar in concept to the attenuated whole infectious agents used to successfully prevent so many viral and bacterial infectious diseases. This doubt was not due to concern about the potential safety of such an attenuated *P. falciparum* sporozoite vaccine. Rather, it was believed to be impossible to manufacture and administer adequate quantities of aseptic, purified, well-characterized, stable *P. falciparum* sporozoites that met regulatory and cost of goods requirements.^{18,19} Therefore, instead of directly pursuing the live attenuated sporozoite vaccine approach, sera and T cells from volunteers immunized by the bite of irradiated *P. falciparum* sporozoite-infected mosquitoes were used to try to characterize the immune responses and identify target antigens that were responsible for the protective immunity.²⁰⁻³⁰ The hope was that these findings would lead to development of an effective subunit vaccine.

Sanaria was founded to overcome the perceived obstacles to the development and commercialization of a metabolically active, non-replicating *P. falciparum* sporozoite (PfSPZ) vaccine. At the outset it was thought that the chance of success was good for several reasons.³¹

- The immunogen, radiation-treated PfSPZ, was already identified and known to be highly protective in humans.
- Development of the vaccine was therefore not a molecular biology and antigen discovery problem. Instead, manufacturing a PfSPZ vaccine presented a completely novel set of challenges involved with manufacturing a vaccine for the first time in mosquitoes, controlling all of the elements of the manufacturing process, and developing unique assays to characterize the vaccine.
- Since a large, profitable traveler's market for the vaccine was anticipated, a potential mechanism existed for raising the

estimated \$0.5 to \$1.0 billion required to develop, register and deploy the vaccine to benefit the primary target population, infants in sub-Saharan Africa.

Sanaria's vaccine, SanariaTM PfSPZ Vaccine, was first administered to volunteers in clinical trials in May 2009. Achieving this milestone required several years of research, more than a year of process development, establishment of the world's first manufacturing facility for a metabolically active, non-replicating (live but incapable of causing disease) malaria vaccine, manufacturing in compliance with current Good Manufacturing Practices (cGMPs), development and approval of a clinical protocol, and preparation and successful submission to the U.S. Food and Drug Administration (FDA) of an investigational new drug application (IND). The steps in the development of the SanariaTM PfSPZ Vaccine are described below. In addition, plans are outlined for optimizing the process of reaching the stage of submitting a successful Biologics License Application (BLA) and launching the vaccine.

Development of the PfSPZ Vaccine

Research. When Sanaria was founded there were three major questions that had to be addressed regarding a PfSPZ vaccine.³¹ Could one (1) administer the vaccine by a route that was clinically appropriate; (2) produce adequate quantities of the vaccine; and (3) manufacture a PfSPZ vaccine that met regulatory and cost of goods requirements?

Administration of the vaccine by a clinically appropriate route. One of the first Sanaria studies addressed whether immunization and protection of rodents could be achieved when radiation-attenuated *P. yoelii* sporozoites (IrrPySPZ) were inoculated by the subcutaneous (SC) route. 100% protection was achieved.³² Subsequently it was found that protection could also be achieved by administration through intradermal (ID), and intramuscular (IM) routes. In addition, Sanaria scientists have been able to demonstrate that 90%–100% of mice can be protected by immunization with previously cryopreserved, IrrPySPZ administered by the SC or ID route (unpublished). These studies provide a foundation for determining the optimal route of administration to humans in a clinical trial.

Producing adequate quantities of PfSPZ. It requires the bites of greater than 1,000 irradiated, *P. falciparum* sporozoite-infected mosquitoes to achieve high level protection in humans. Recent data indicate that mosquitoes inoculate 100–300 sporozoites in the dermis when they feed.³³ If this is the case in humans, this would mean that protected volunteers received 100,000 to 300,000 Pf sporozoites during these studies. In the *P. yoelii* (Py) rodent malaria model system it requires immunization with 2–4 times more cryopreserved, IrrPySPZ than fresh, irradiated PySPZ, and 2–4 times more IrrPySPZ administered intradermally than intravenously to achieve similar levels of protection. This could mean that it may take 4–16 times more cryopreserved PfSPZ administered intradermally or subcutaneously than administered by mosquito bite to achieve the same level of protection. Until the number of PfSPZs per dose and the dosage regimen are finally established, it will be impossible



Figure 1. Dissection of salivary glands from *P. falciparum* sporozoite-infected mosquitoes. This process is carried out under aseptic conditions in compliance with current Good Manufacturing Practices in Sanaria's clinical manufacturing facility (Photo Sanaria Inc.).

to know what resources will be required to produce adequate quantities of PfSPZ.

Nevertheless, one early concern was whether dissection of salivary glands from mosquitoes might be limiting. Currently, a six-person dissection team (Fig. 1) can remove the salivary glands from more than 500 mosquitoes per hour. Assuming one dose of vaccine per mosquito, this small team, working in a pilot manufacturing facility can produce more than 500 doses of vaccine per hour. When we establish the final dose and dosage regimen, the hourly output of vaccine required from the dissection team can be determined. However, current data indicate that it will be feasible to produce quantities of the Sanaria™ PfSPZ Vaccine adequate for anticipated requirements.

Manufacture Sanaria™ PfSPZ Vaccine to meet regulatory requirements. At a minimum, the Sanaria™ PfSPZ Vaccine must be (1) free of contaminating pathogens (i.e., aseptic), (2) free of significant amounts of mosquito-derived material (i.e., pure), (3) non-replicating (i.e., attenuated) and (4) capable of eliciting a protective immune response (i.e., potent). Sanaria scientists have developed methodologies, equipment, and standard protocols for producing the Sanaria™ PfSPZ Vaccine by an aseptic process that yields pathogen-free sporozoites as determined by standard FDA-mandated assays. Methods to remove contaminating mosquito material from the PfSPZ and an assay to measure such material have also been developed. Working with the US National Institute of Standards and Technology (NIST), Sanaria has developed a dosimetry-based monitoring system that measures

the minimum and maximum possible dose of irradiation that each mosquito receives. Having established the minimum dose of irradiation that attenuates all parasites, the results of the dosimetric monitoring together with an in vitro attenuation assay developed by Sanaria, ensure that all sporozoites are adequately attenuated. Finally, Sanaria has established an in vitro potency assay. With these methodologies now established to produce vaccine that was free of pathogens, uncontaminated by significant amounts of mosquito material (Fig. 2), adequately attenuated, and potent, the next step was to implement a cGMP-compliant manufacturing process.

Process development. Component procedures to produce the vaccine were studied with considerations given to yields, quantities, quality, speed, timing, adaptability to scale and suitability for conformance to manufacture under cGMPs. These component procedures were then integrated to generate a single coordinated process that included producing aseptic gametocyte cultures and aseptic mosquitoes, feeding gametocytes to mosquitoes, maintaining infected aseptic mosquitoes, irradiating infected mosquitoes so that the PfSPZ could not replicate, harvesting irradiated PfSPZ from the mosquitoes, removing salivary gland material from the harvested sporozoites without a major reduction in potency or numbers of PfSPZ, formulating bulk PfSPZ preparations, and cryopreserving PfSPZ. In-process assays that monitor the performance and aseptic integrity of the process at multiple steps during production were developed and implemented. Release assays that characterize and describe bulk PfSPZ preparations and final

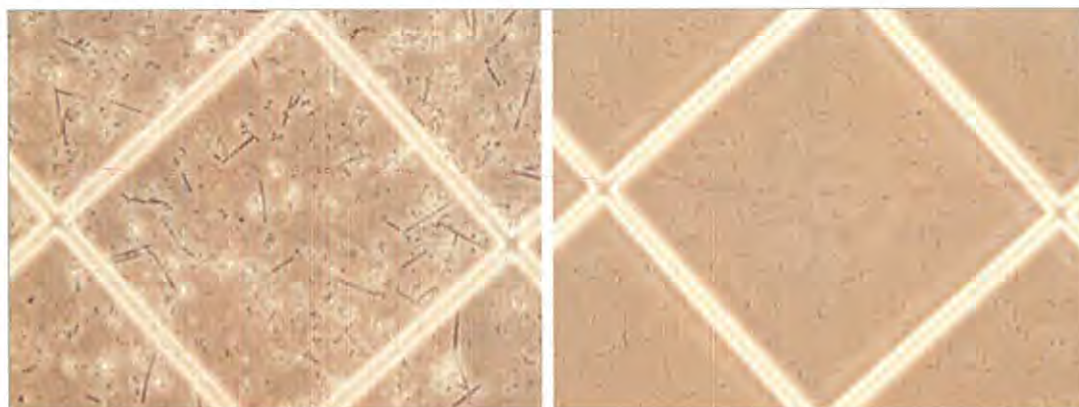


Figure 2. Purified PfSPZ. The purification process significantly reduces the amount of salivary gland and mosquito material contaminating the PfSPZ. In the photomicrographs the numbers of PfSPZ in the Pre- and Post-Purification preparations were approximately 17×10^6 PfSPZ/mL (200X magnification) (Photo Sanaria Inc.).

Sanaria™ PfSPZ Vaccine were also developed and implemented. More than 10 integrated production campaigns designed to consolidate and document the capability of the manufacturing process were conducted during 2006. Quality systems and manufacturing teams, together with external consultants, established the standard operating procedures and batch records required for manufacture in compliance with cGMPs.

Manufacturing under current cGMPs. *Sanaria™ PfSPZ Vaccine lots for pre-clinical studies in support of the IND application.* After establishing the manufacturing process and associated documentation, Sanaria conducted multiple production campaigns in 2007 to manufacture and release Sanaria™ PfSPZ Vaccine lots for pre-clinical toxicology and immunology studies in support of an IND application. The production campaigns were completed successfully and several lots were released for repeat dose toxicology studies and biodistribution studies in animals.

Sanaria™ PfSPZ Vaccine manufactured for clinical trials. The facility where Sanaria™ PfSPZ Vaccine was manufactured for animal toxicology and biodistribution studies was considered unsuitable for the manufacture of material intended for clinical trials. Indeed, Sanaria's first production facility was described as occupying a "...dismal mini-mall in suburban MD".³⁴ In partnership with the PATH-Malaria Vaccine Initiative (PATH-MVI), a grant was obtained from the Bill and Melinda Gates Foundation to support vaccine development efforts. A portion of these funds was used to build the world's first facility for manufacturing a live, attenuated malaria vaccine. In the spring and summer of 2008 multiple clinical lots of Sanaria™ PfSPZ Vaccine were manufactured and released at this new facility. They are assessed as part of an ongoing stability program, and have been shown to be stable.

Regulatory. Many colleagues in the field had suggested that even if the problems of manufacturing the vaccine could be overcome, it would be difficult, if not impossible to meet regulatory requirements. Sanaria began informal interactions with the FDA in 2003 and formal interactions in 2005. All FDA recommendations and directives have been implemented. The IND

was submitted to the FDA in February 2009, and Sanaria was allowed to proceed to clinical trials 30 days later.

Design of clinical protocol and execution of clinical trial. Development and finalization of the clinical protocol and approval by the investigational review boards (IRBs) was a lengthy process and involved consultation with Sanaria's advisory board (www.sanaria.com) and a vaccine advisory committee composed of internationally renowned experts in the fields of vaccine development, clinical vaccinology and malariology. The primary goal of the first clinical trial is to establish that the vaccine is safe and well tolerated. The immunogenicity (antibody and T cell responses) of the vaccine is also being assessed. However, volunteers can be safely infected with *P. falciparum* by the bite of mosquitoes transmitting live, infectious sporozoites.³⁵ Thus, recognizing that the dosage regimen is not based on any clinical experience, and may be sub-optimal, the protective efficacy of the vaccine against experimental challenge in malaria-naïve volunteers will also be determined. The trial is a dose-escalation study using two routes of administration, subcutaneous (SC) and intradermal (ID) (Fig. 3). The study is a randomized, dose escalation study in which volunteers receive 4-6 doses of Sanaria™ PfSPZ Vaccine containing 7,500, 30,000, or 135,000 sporozoites administered either SC or ID at 4-week intervals, and are challenged three weeks after the last dose of vaccine by the bite of five mosquitoes infected with *P. falciparum* sporozoites. Recruitment began in April 2009 at the Naval Medical Research Center and the University of Maryland Center for Vaccine Development, both in MD, USA. The first inoculation was administered in late May 2009 and the study will run through July 2010.

Next Steps

Goal. Our goals are to achieve successful submission of a BLA, commercial launch of the vaccine, and use by the most malaria-afflicted populations of a Sanaria™ PfSPZ Vaccine as soon as possible. Achievement of these goals will require an extraordinary effort and cooperative interaction among partners throughout the world. Our plan is in its early stages, but

some of the required and proposed efforts and interactions are summarized below.

Clinical development plan. Sanaria's primary mission since its inception has been to develop and deploy a vaccine that prevents *P. falciparum* infection and thereby *P. falciparum* malaria-associated severe illness and death in the most affected populations, infants and young children in Africa. To achieve its mission Sanaria has always considered there to be two primary, quantifiable markets for an attenuated Sanaria™ PfSPZ Vaccine,³¹ the African infants who bear the greatest burden of disease and travelers from the developed world, income from whom would support optimal deployment of the vaccine to African infants. This has always meant aiming for immunizing infants in the Expanded Program for Immunization (EPI) of the World Health Organization (WHO) to achieve the primary mission.

Sanaria's primary mission has not changed, but the global strategy for combating malaria is changing, and thus, the approach to achieving the mission is likely to be changed accordingly. The investment of billions of dollars in malaria control efforts is having a significant positive impact,³⁶⁻⁴⁰ reducing the transmission of malaria in some areas, including some sites in sub-Saharan Africa. This has allowed for consideration of elimination of *P. falciparum* from defined geographic regions and even contemplation of the eventual eradication of malaria (<http://www.rbm.who.int/gmap/gmap.pdf>). Based on this perspective, two important considerations affect the way a highly effective sporozoite vaccine might be used. First, as malaria transmission decreases and acquired immunity wanes, the demographics of malaria disease will change to include older age groups, and increasing numbers of older children will require vaccination outside the EPI. Second, Sanaria and some of its partners now consider mass immunization of the entire population in a defined geographic area to eliminate *P. falciparum* from the region to be a third major indication (market) for a vaccine of this type.⁴¹ This additional market has prompted us to refine the clinical development plan so it additionally supports delivering the vaccine in campaign-based mass administration programs to most of the population (see below).

Malaria vaccine development benefits from two key advantages not shared by vaccines for most other neglected diseases and diseases of poverty. First, a safe, reproducible experimental challenge system allows for multiple, relatively small studies in volunteers in the developed world. Second, because there is a developed world market, one can anticipate licensure of the vaccine for use in these countries by regulatory authorities like the FDA, the European Medicines Agency (EMA), and comparable institutions around the world. We have reasoned from the outset that such approval for use in the developed world will speed deployment and widespread uptake of the vaccine in the developing world.³¹ Thus, we plan to move forward with an integrated clinical development plan. The goal is to achieve licensure of the vaccine in the developed world for all non-pregnant individuals except young infants, followed rapidly by licensure in the developing world, first for the same populations, and then for young infants and pregnant women. This clinical development pathway will involve studies both in experimentally challenged volunteers



Figure 3. Intradermal injection of Sanaria™ Vaccine (Photo US Navy, taken by Mass Communication Specialist 3rd Class Timothy Wilson)

in non malaria-endemic regions, and in naturally exposed populations in malaria endemic regions of the world, especially Africa. Licensure of the vaccine for infants and pregnant women in the developing world would eventually support extension of licensure in the developed world to these same groups, such that the vaccine would ultimately be licensed for use in all individuals with potential exposure to malaria.

Experimentally challenged volunteers. It is anticipated that further studies in immunized, experimentally challenged volunteers in the USA, the Netherlands, Colombia and other countries will be conducted to optimize the route and method of administration, number of doses, interval between doses, volume administered, and site of administration. In addition, the longevity of protection, protection against different geographic isolates of *P. falciparum*, and protection against *P. vivax* will all be determined in these immunized, experimentally challenged, malaria-naïve volunteers. The goal is to achieve high-level protection with the fewest numbers of doses of vaccine and the fewest numbers of PfSPZ per dose.

Naturally exposed populations in malaria-endemic regions. The primary target population is the 25 million babies born annually in sub-Saharan Africa. Another important target population is pre-adolescent girls, in order to prevent malaria in pregnancy, which is associated with increased maternal morbidity and mortality, spontaneous abortion, and low birth weight infants who are at increased risk of dying in the first year of life. In order to progress to testing the vaccine in these populations as rapidly as possible, trials will be conducted in sub-Saharan Africa (and potentially other malaria-endemic regions of the world), as soon as vaccine safety has been demonstrated in the first clinical trial now underway in the USA in healthy, malaria-naïve adults. The initial goal of these trials will be to demonstrate that the vaccine is safe and immunogenic in malaria-experienced adults as well. The clinical development process will then move rapidly to assessment of the vaccine safety, immunogenicity, and efficacy in the populations that suffer the most from malaria caused by *P. falciparum*.

Following demonstration of acceptable vaccine safety and reactogenicity in malaria-experienced adults, the next step in the

clinical development pathway will be to test the safety and efficacy of the Sanaria™ PfSPZ Vaccine in children at high risk of malaria infection and disease. While evidence of efficacy against infection may be demonstrated by initial trials in semi-immune adults, the pediatric trials will provide the critical proof of concept that the Sanaria™ PfSPZ Vaccine prevents natural infection and clinical malaria, establishing a powerful rationale for developing the vaccine as a licensed product to control and eventually to help eliminate malaria. Moreover, these trials will demonstrate whether the Sanaria™ PfSPZ Vaccine provides protective immune responses against genetically diverse malaria parasites found in nature, shaping the direction of the clinical development strategy. While there is evidence of heterologous protection from early experimental challenge studies (reviewed in ref. 12), confirming protection against genetically diverse parasites through natural infection is a critical step in the early clinical development of the Sanaria™ PfSPZ Vaccine. If, for example, the vaccine shows very high efficacy in malaria-naïve North American volunteers experimentally challenged with the same strain as that used in the vaccine, but initial efficacy studies in African children demonstrate significantly lower efficacy, it will be important to assess the contribution, if any, of parasite genetic diversity to this reduced efficacy.⁴² Understanding and overcoming any allelic restriction of vaccine efficacy, possibly through the development of a multi-strain attenuated Sanaria™ PfSPZ Vaccine, could emerge as a leading priority in the further development of this vaccine. If, however, high efficacy, similar to that anticipated in homologous challenges, is demonstrated against diverse natural parasites, the clinical development plan can concentrate on optimizing dose and delivery of the single strain vaccine and taking this formulation forward to licensure. It may also be possible that the vaccine will show greater efficacy in African populations continuously exposed to the bite of *P. falciparum* sporozoite-infected mosquitoes as compared to malaria naïve North American adults, as it is likely that even during the immunization process boosting of vaccine-induced immunity by natural exposure to sporozoites through the bite of infected mosquitoes will occur. Following initial proof of concept in the target population, a Phase 2 testing program conducted in a variety of malaria-endemic settings with different transmission intensities and seasonality will assess impact of natural infection on the immunogenicity and efficacy of the Sanaria™ PfSPZ Vaccine, as well as optimize the dosing regimens and delivery systems.

Optimizing efficiency of manufacture and scale up. Based on the results of multiple successful production campaigns under cGMPs, which have manufactured Sanaria™ PfSPZ Vaccine for the first clinical trials, Sanaria has identified specific opportunities for further optimization of its manufacturing process and control assays. In the next two years the Sanaria team will be conducting innovative research and development to establish and finalize a more efficient, scaled-up, manufacturing process and set of control assays necessary to take the Sanaria™ PfSPZ Vaccine forward. To address this overarching goal, Sanaria will aim to reduce the quantity of blood products and personnel necessary for the production of gametocytes to feed to mosquitoes, increase the efficiency of mosquito production, increase the numbers of

sporozoites produced in and harvested from infected mosquitoes, and increase the size of vaccine lots. This increased efficiency will be accompanied by scale-up of the entire manufacturing process. An essential element of the work will be that the assays for in process control, release and stability will be optimized and then validated for subsequent production of Sanaria™ PfSPZ Vaccine. Success in the next two years will leave Sanaria ideally placed to design and construct a facility in which the Sanaria™ PfSPZ Vaccine can be optimally manufactured for pivotal Phase 3 studies, licensure and commercial launch.

Optimizing and validating assays. *Quality control of the Sanaria™ PfSPZ Vaccine.* Assays used for release and stability of the Sanaria™ PfSPZ Vaccine are required to demonstrate that the vaccine is free of contaminating pathogens (aseptic), free of contaminating mosquito material (pure), unable to cause malaria in recipients (non-replicating), and capable of inducing a protective immune response in recipients (potent). Assays that demonstrate the vaccine is free of microbial contaminants are harmonized compendial methods. However, assays for purity, attenuation (non-replicating), and potency of the Sanaria™ PfSPZ Vaccine are unique. Sanaria scientists have developed assays that have been adequate for an IND, but plan to improve the performance characteristics of these assays and validate them prior to licensure.

Prediction of vaccine efficacy in an individual. It will be important to develop and validate one or more immunological assays that predict protective immunity following vaccination. Based on animal studies, the protective immunity engendered by immunization with radiation-attenuated sporozoites is thought to be mediated primarily by T cells that recognize and eliminate parasite-infected hepatocytes, and secondarily by antibodies against sporozoites that prevent hepatocyte invasion and normal parasite development.⁴³⁻⁴⁵ Work is underway in-house as well as in collaboration with the Naval Medical Research Center and the Vaccine Research Center at NIAID to develop an assay that, at a minimum indicates that an individual is protected and will ideally be based on the mechanism of protective immunity.

Minimizing number of doses and total dosage of PfSPZ required to induce greater than 90% protection. All available data indicate that greater than 90% protection against *P. falciparum* infection in humans can be achieved by immunization with radiation-attenuated *P. falciparum* sporozoites, if adequate numbers of doses of adequate numbers of sporozoites are administered. However, the number of PfSPZ per dose and the dosage regimen, including number and spacing of doses, remain to be established. In studies of volunteers immunized by the bite of irradiated, *P. falciparum* sporozoite infected mosquitoes, all protected volunteers received at least five primary immunizing biting sessions, some received as many as 19 primary immunizations and the median number was between 9 and 10 immunizing biting sessions.¹² Improvements in the method of administration or an increase in potency of a Sanaria™ PfSPZ Vaccine should be able to reduce the number of doses of PfSPZ and the number of PfSPZ per dose and therefore the cost of administering the vaccine and the cost of producing a dosage regimen. For this reason Sanaria scientists are working on optimizing the methods

of administration of metabolically active, non-replicating sporozoites and increasing the potency of individual doses of PfSPZ.

Route and method of administration. The Sanaria™ PfSPZ Vaccine differs from most, if not all, attenuated viral and bacterial vaccines, in that the sporozoites are non-replicating. Attenuated viral and bacterial vaccines are either fully replicative, but attenuated with regard to virulence, or replication deficient. Thus, it cannot be assumed that this non-replicating vaccine can be administered like other attenuated vaccines. Furthermore, we know that at least twice as many cryopreserved irradiated PySPZ administered to mice by needle and syringe by the ID, SC or IM routes are required to achieve 90%–100% protection compared to irradiated PySPZ administered by the intravenous (IV) route, and more doses of sporozoites are required to achieve the same level of protection. Furthermore, varying the anatomical site where ID or SC immunizations are made has a significant impact on immunogenicity and protective efficacy of irradiated sporozoites in animal models. Sanaria's goal is to develop a method of vaccine administration that induces immune responses and protective immunity comparable to that achieved by IV administration of metabolically active, non-replicating sporozoites. To this end, Sanaria is working in animal models to identify approaches that achieve this goal and has also established collaborations with a number of groups to determine whether administration of sporozoites by specific devices can enhance immunogenicity and protective efficacy. Ultimately, an optimized method of vaccine administration for humans can only be identified and established in clinical trials.

Potency of metabolically active, non-replicating sporozoites. IV administration of three doses of just 375–750 (total dose = 1,125–2,250 irrPySPZ) freshly dissected, irrPySPZ protects 90%–100% of mice against challenge with fully infectious PySPZ.⁴⁶ The same level of protection is achieved with IV administration of three doses of 1,500 (total dosage = 4,500 irrPySPZ) purified, cryopreserved IrrPySPZ (unpublished). The dosage of attenuated IrrPySPZ required to achieve complete protection even when cryopreserved is therefore quite low (4,500 total sporozoites). Nonetheless, it requires immunization with 2–4 times as many purified, cryopreserved IrrPySPZ to achieve 90%–100% protection as it does with purified, fresh irrPySPZ. This means that the cryopreservation process for *P. yoelii* sporozoites can be improved, and Sanaria scientists are working to improve the cryopreservation process for PySPZ to produce sporozoites that are more potent.

Another approach to improving immunogenicity and protective efficacy of the sporozoite vaccine might be to include an adjuvant in the formulation. In general, adjuvants are used to enhance immune responses to “dead” vaccines, not live attenuated whole infectious agent vaccines. This is in part because adjuvants like aluminum hydroxide, which is essentially the only adjuvant used for licensed vaccines in the USA, MF59, which is licensed in Europe, and adjuvants like the AS0 series of adjuvants used with GlaxoSmithKline Biological's candidate PfCSP malaria vaccine immunogen, RTS,S, would be likely to reduce the capacity of live attenuated vaccines to invade host cells and induce the immune responses required for protection. Importantly, a precedent exists

for the use of an adjuvant to enhance protection conferred by radiation-attenuated sporozoites. The marine sponge-derived glycolipid, α -galactosylceramide (α -GalCer), a ligand for Natural Killer T (NKT) cells, induces robust cytokine production by NKT cells, including IFN γ , and also results in bystander activation of T cells, dendritic cells, and B cells. In 2002, Tsuji and colleagues reported that the use of α -GalCer in conjunction with a suboptimal dose of IrrPySPZ increased protective efficacy of IrrPySPZ by more than 4.5-fold as compared to a sub-optimal dosage regimen of irrPySPZ (from 20% to 93%).⁴⁷ Sanaria scientists are conducting in-house research and collaborating with other institutions to determine whether adjuvants can be used to enhance the protective efficacy of sporozoite vaccines.

Optimizing the logistics for delivery in the field. There are multiple potential primary markets in sub-Saharan Africa for a Sanaria™ PfSPZ Vaccine. These include: (1) the 25 million infants born annually (prevention of severe morbidity and mortality in infants and children), (2) the 7.5–10 million girls that reach adolescence annually (prevention of complications of malaria in pregnancy and neonatal and infant mortality due to malaria-caused low birth weight), (3) older children, adolescents and adults who will become increasingly at risk of severe disease as transmission intensity is reduced by ongoing control measures, (4) travelers from non-endemic to endemic regions within Africa, and (5) large portions of the entire 800 million population of sub-Saharan Africa should mass immunization programs be instituted to eliminate *P. falciparum* from defined geographic areas.

A high proportion of newborns in many areas are already immunized with other vaccines within the EPI. However, the other groups are not currently reached with high coverage by any vaccination program. The Sanaria™ PfSPZ Vaccine is currently cryopreserved, stored and distributed in liquid nitrogen vapor phase (LNVP). Delivery of a vaccine in LNVP has significant potential advantages over delivery methods that use cold chains of -20°C (for example, the varicella and live attenuated flu vaccines) or 2–8°C (most EPI vaccines). These advantages include elimination of specialized and time-sensitive packaging, the need for refrigerated vehicles, and the need for electricity during delivery and at delivery sites and a significantly longer stability of the vaccine without loss of potency. Independence from conventional refrigerators and freezers and the use of LNVP dry shipper containers, which perform double duty as both the shipping/transportation containers and as mobile storage units for vaccine while it is being administered, provides a level of flexibility, efficiency and cost saving in vaccine distribution not possible with conventional cold chains.

An extensive network of manufacturers and suppliers of liquid nitrogen is well established in sub-Saharan Africa and LNVP transportation is used routinely for biological sample collection, blood storage and transfusion, and artificial insemination. Moreover, a number of live attenuated veterinary vaccines are maintained and distributed in LNVP or liquid nitrogen, including the live, whole organism *Theileria parva* sporozoite vaccine, which is used to protect cattle against the tick-borne apicomplexan parasite that causes East Coast Fever. This vaccine has been successfully used in a country-wide campaign in Tanzania.^{48,49}

Experimental human dendritic cell and anticancer vaccines are also cryopreserved in LNVP, but the Sanaria™ PfSPZ Vaccine would be the first licensed human live anti-infectious agent vaccine cryopreserved in LNVP.⁵⁰⁻⁵²

For immunizing infants, distribution of cryopreserved Sanaria™ PfSPZ Vaccine would ideally be done in a way that is complementary to the EPI program. Integration with EPI vaccines will be facilitated if malaria-endemic country EPI programs shift to the more efficient hub and spoke, privatized vaccine delivery system that has recently been adopted in the United States and Thailand. For immunization of early adolescent females and the rest of the population on an ongoing basis, and for mass immunization of all age groups in defined geographic areas, target groups for which there is no established vaccine delivery infrastructure, a LNVP delivery system has major potential advantages over delivery of a vaccine that requires a cold chain dependent on electricity. We have already performed a pilot demonstration of the effectiveness of a LNVP cold chain by shipping the Sanaria™ PfSPZ Vaccine from the U.S., in collaboration with the Noguchi Memorial Institute for Medical Research, to the Navrongo Health Research Centre in northern Ghana and back (a distance of over 14,000 miles) with no loss of potency. In the coming years we will be working with national governments, international organizations, and private sector companies to ensure that a highly protective Sanaria™ PfSPZ Vaccine cryopreserved in LNVP can be delivered to every individual who needs it.

Thermostabilization of Sanaria™ PfSPZ Vaccines. Despite the potential advantages of a vaccine stabilization and delivery system based on LNVP, and the possibility that vaccines and other biologicals may in the future be delivered in Africa by the hub and spoke delivery system that we advocate for delivery of a vaccine in LNVP, it is prudent to investigate alternative methods of stabilization that would allow for delivery of the Sanaria™ PfSPZ Vaccine in the system and cold chain already established by the EPI for delivery to African infants. This means stabilization of a Sanaria™ PfSPZ Vaccine, minimally at -20°C (temperature of a standard freezer), more optimally at 2–8°C (temperature of a standard refrigerator), and eventually at ambient temperature. Because of the potential importance of and difficulty in thermostabilization of the Vaccine Final Product (VFP), this goal is being pursued through in-house research and extensive external collaborations.

Sanaria has collaborated to develop high throughput screening methods, and is now working to optimize those methods. Thermostabilization of a number of viral and bacterial vaccines by lyophilization is possible, but no vaccines composed of live eukaryotic cells, such as *P. falciparum* sporozoites, are thermostabilized by any method other than by cryopreservation. Routine lyophilization of eukaryotic cells has been limited to platelets and mammalian erythrocytes which are anucleate and which retain only a proportion of their activity following reconstitution. One of the challenges of lyophilization is achieving the presence inside the cell, at relatively high concentrations, of lyoprotective agents (LPAs), usually sugars, and removal of almost all cellular water.

In addition to an in-house program on lyophilization, Sanaria is working with partners to explore other approaches. These include lyophilization, foam drying,⁵³ spray drying,⁵⁴ and ambient

dessication.⁵⁵ Foam drying is essentially lyophilization by boiling the product at a temperature just above the freezing point of the suspending medium in a partial vacuum. Spray drying involves the use of protective compound(s) introduced at relatively high concentrations into a liquid formulation surrounding the cells and the removal of most cellular water. The presence of internal LPAs may also be necessary. Water removal occurs extremely rapidly during brief exposure of cells to a jet of high temperature nitrogen gas. The process of ambient desiccation utilizes high concentrations of LPA-type compounds and low relative humidity to produce a dried product over a period of a few hours.

It is anticipated that during the next few years one or more of these approaches to thermostabilization of VFP will be optimized and fully characterized. Based on the experience with viral and bacterial vaccines, it is unlikely that a Sanaria™ PfSPZ Vaccine thermostabilized by one of the approaches under development will have the long-term stability or potency of a Sanaria™ PfSPZ Vaccine cryopreserved in LNVP. This means that the total number of PfSPZ required to achieve the same level of protection will likely be significantly greater than when PfSPZ are stabilized in LNVP. Thus, when such an approach has been optimized and characterized, a decision will have to be made regarding whether to move from the Sanaria™ PfSPZ Vaccine cryopreserved in LNVP to one thermostabilized by one of these other methods. If significant improvements can be made in the efficiency of manufacture and scale up of the Sanaria™ PfSPZ Vaccine, and the numbers of PfSPZ per dose and the numbers of doses of PfSPZ can be reduced, it will be easier to make the transition to a form of thermostabilization that requires more sporozoites per dose to achieve high level protection:

Comparative assessment of safety and efficacy of PfSPZ attenuated by irradiation, gene deletion or chemical exposure. The development of the radiation-attenuated Sanaria™ PfSPZ Vaccine is based on observations first made in mice.⁵⁶ These studies led to the demonstration that exposure of human volunteers to the bite of mosquitoes carrying radiation-attenuated PfSPZ protected humans against challenge with infectious PfSPZ.¹² It has now been demonstrated that immunization of mice with genetically attenuated *P. berghei* or *P. yoelii* rodent malaria sporozoites,⁵⁷⁻⁶⁵ and with chemically attenuated *P. berghei* sporozoites^{66,67} protects mice against challenge with infectious sporozoites. Genetically altered (knock out) *P. falciparum* parasites that give rise to attenuated sporozoites have been engineered by teams including scientists from the Radboud University Nijmegen Medical Centre, the University of Leiden, and Sanaria,⁶⁸ and from the Seattle Biomedical Research Institute (SBRI) and the Walter and Eliza Hall Institute.^{68,69} It is anticipated that by the end of 2010 a study to be carried out as a collaboration between SBRI and the U.S. Military Malaria Vaccine Program will be completed to determine if exposure of volunteers to the bites of mosquitoes carrying genetically attenuated *P. falciparum* sporozoites is safe and protective. The manufacturing process for PfSPZ is likely to be identical regardless of whether the sporozoites are attenuated by irradiating, knocking out genes, or treating with chemicals. While we currently have human data only for radiation-attenuated PfSPZ, and have only manufactured under cGMPs a radiation-treated Sanaria™ PfSPZ Vaccine, during

the next few years we intend to generate comparative data that will enable us to begin to determine whether genetically or chemically attenuated PfSPZ offer any advantages over radiation-attenuated PfSPZ with respect to safety, manufacturing processes, protective efficacy, or cost.

Radiation-attenuated PfSPZ are non-replicating, as are the first generation genetically attenuated sporozoites that will be assessed in humans.⁶⁹ They stop development at the early liver stage. It is now possible to produce genetically altered parasites that develop to, (1) later liver stages and then stop development before achieving maturity (replication deficient at the liver stages), or mature liver stages that produce infectious merozoites that arrest in erythrocytes (replication competent at the liver stage, but non-replicating at the disease causing erythrocytic stage). As compared to the non-replicating, radiation attenuated and first generation genetically attenuated sporozoites, these replication deficient and replication competent parasites will definitely produce more parasite material in an immunized individual (in essence an increase in dose) and will produce antigens not expressed by the non-replicating parasites (wider range of targets of protective immune responses). Thus, it is possible that genetically altered, replication deficient or replication competent (at the liver stage) parasites could induce protective immune responses at a lower dose (numbers of sporozoites) to a wider array of target antigens than a non-replicating parasite. This will need to be investigated.

Summary

A vaccine that provides sterilizing immunity against infection with *P. falciparum* will prevent infection, malaria disease and parasite transmission. This type of vaccine would be ideal for reducing *P. falciparum* malaria-associated morbidity and mortality when administered to infants and children, adolescent girls prior to pregnancy and other at risk groups, and eliminating *P. falciparum* from defined geographic areas when administered to all age groups in a mass administration campaign. Rodent and human data indicate that the best current candidate immunogen for such a vaccine is metabolically active, non-replicating PfSPZ. Developing a vaccine based on attenuated PfSPZ was not previously pursued, because it was inconceivable to scientists in the field that a vaccine based on PfSPZ could be manufactured and released in compliance with

cGMPs. The Sanaria™ PfSPZ Vaccine has now been manufactured and released, cleared for testing in clinical trials by the FDA, and has entered into Phase 1 clinical testing. Herein we have outlined how the Sanaria™ PfSPZ Vaccine arrived at this stage of development, current challenges, and how we anticipate moving an attenuated Sanaria™ PfSPZ Vaccine forward to licensure, commercialization and deployment.

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